BRIEF COMMUNICATIONS

2-Amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine, a Carcinogen in High-Temperature-Cooked Meat, and Breast Cancer Risk

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Although intake of well-done red meat has been associated with an increased risk of breast cancer *(1),* it is unclear what component(s) of well-done red meat is associated with this risk. Meats cooked to well-done at high temperatures contain heterocyclic amines (HCAs), such as 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline (DiMeIQx), 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx), and 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) *(2–9).* The amounts of these compounds vary according to cooking technique, temperature, cooking time, and type of meat *(10,11).* Although PhIP administered orally can induce mammary gland carcinomas in rats *(12–14),* the association of HCAs with human breast cancer is unclear. Two studies have investigated the association between meat-cooking methods and breast cancer. One study *(15)* did not obtain information on the degree of meat doneness, from which levels of HCA can be estimated, and the other study *(16)* used HCA estimates from laboratory-cooked meat samples from one country and subjects from a different country.

We used a newly created database [for details, *see (10)* and *(11)*] to estimate HCAs in a breast cancer case–control study of 41 836 cohort members participating in the Iowa Women's Health Study *(1).* Of the selected subjects, 273 case patients (60% of all women with breast cancer diagnosed from 1992 through 1994) and 657 control subjects (75% of randomly selected cohort members who were alive and free of cancer on January 1, 1992, and participated in the 1992 follow-up survey) participated in this study. All subjects completed a self-administered food-frequency questionnaire that included validated questions on frequency of intake and cooking techniques of 15 meat items. The participants reported their usual preference for level of doneness by using a series of color photographs that represented increasing levels of doneness of a hamburger patty and beef steak (four photographs for each) as well as bacon (three photographs) *(10,11).*

We estimated HCA intake by use of our database *(10,11,17)* and the responses from the food-frequency questionnaire. First, we estimated gram consumption by frequency, portion size, cooking technique, and doneness level. Second, we derived HCA intake by multiplying grams of meat by the HCA concentration measured for each cooking technique/doneness level for that meat type and summed across the three meats. To estimate the doneness levels, we added the gram amounts for "rare/medium," "well-done," and "very well-done" steak, hamburger, and bacon. Dietary intakes of each HCA and each type of red meat were lower among the control subjects than among the case patients, with the case patients consuming 50% more PhIP (Table 1).

Table 1. Heterocyclic amines (HCAs) and meat intake in case patients and control subjects*

	Case patients (n = 273)†	Control subjects (n = 657)†
HCA		
DiMeIQx, ng/d‡	2.0 ± 5.6 (0, 2.0, and 4.0)	1.6 ± 2.8 (0, 0.6, and 4.2)
MeIQx, ng/d‡	30.8 ± 52.5 (0.5, 14.3, and 70.2)	24.4 ± 33.8 (0.6, 12.8, and 63.5)
PhIP, ng/d‡	55.7 ± 113.5 (0, 20.4, and 130.6)	36.6 ± 61.3 (0, 13.6, and 94.9)
Meat type, red meat with doneness photographs, g/d§	24.8 ± 19.9 (5.1, 19.6, and 51.3)	23.6 ± 22.1 (3.3, 16.7, and 49.0)
Rare/medium	14.8 ± 16.9 (0, 9.3, and 38.7)	15.3 ± 18.6 (0, 9.3, and 39.0)
Well-done	6.7 ± 12.8 (0, 1.3, and 21.0)	6.5 ± 13.2 (0, 1.1, and 21)
Very well-done	3.3 ± 9.7 (0, 0, and 9.1)	1.8 ± 6.1 (0, 0, and 4.6)

*Age (years) distribution, mean (10th, 50th, and 90th percentiles): case patients = 61.4 years (56, 61, and 67 years); control subjects = 60.9 years (56, 61, and 67 years).

†Values = mean ± standard deviations (10th, 50th, and 90th percentiles).

‡DiMeIQx = 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline; MeIQx = 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline; PhIP = 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine.

§Red meat in this study consists of steak, hamburger patty, and bacon.

Odds ratios (ORs) were computed with unconditional logistic regression *(18),* with test of trends based on continuous variables (Table 2). The association between HCA and risk was determined for each HCA individually and with adjustment for the other HCAs. ORs for DiMeIQx, MeIQx, and PhIP are presented in two ways: increments of 10 ng/day and categorically. ORs associated with an increase of 10 ng/day in daily consumption of HCAs provide the relative potency of the different HCAs and make it easier to compare the results between studies with different populations, where the amounts consumed differ.

We observed an increased risk of breast cancer across increasing quintiles of PhIP consumption; ORs were 1.0 (referent), 1.1 (95% confidence interval [CI] = 0.6–1.8), 1.2 (95% CI = 0.7–1.9), 1.4 (95% CI = 0.8–2.3), and 1.9 (95% CI = 1.1–3.4) adjusted for the intake of MeIQx and DiMeIQx, with a P for trend of <.001 (Table 2). MeIQx and DiMeIQx were not associated with risk of breast cancer in these analyses. The levels of HCA intakes are measured with error. If measurement errors are the same for case and control subjects, this generally creates a bias toward the null.

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See "Notes" following "References."

Table 2. Breast cancer risk and heterocyclic amine (HCA) intake analyzed as continuous and categorical variables

	Continuous analysis	
	Individual HCAs in the models, OR† (95% confidence interval [CI])	Individual HCA adjusted for the other two HCAs, OR‡ (95% CI)
	Continuous analyses per 10-ng increments	
HCA*		
DiMeIQx, ng/d	1.32 (0.93–1.86)	0.61 (0.32–1.17)
MeIQx, ng/d	1.03 (1.00–1.07)	1.00 (0.94–1.17)
PhIP, ng/d	1.03 (1.01–1.05)	1.05 (1.02–1.08)

		Categorical analysis	
		Individual HCAs in the models, OR† (95% confidence interval [CI])	Individual HCA adjusted for the other two HCAs, OR‡ (95% CI)
HCAs quintiles§	No. of cases¶	Quintile analyses	
DiMeIQx, ng/d			
0–0.01	51	1.0 (referent)	1.0 (referent)
0.02–0.39	62	1.1 (0.7–1.8)	1.1 (0.7–1.8)
0.40–1.00	43	0.8 (0.5–1.3)	0.7 (0.4–1.2)
1.01–2.41	59	1.1 (0.7–1.7)	0.9 (0.5–1.5)
2.42–30.84	58	1.0 (0.7–1.7)	0.8 (0.4–1.5)
		P for trend‖ = .12	*P* for trend‖ = .14
MeIQx, ng/d			
0–3.0	48	1.0 (referent)	1.0 (referent)
3.1–8.4	45	1.0 (0.6–1.6)	1.0 (0.6–1.8)
8.5–18.0	63	1.3 (0.8–2.1)	1.3 (0.8–2.3)
18.1–35.8	54	1.1 (0.7–1.8)	1.0 (0.6–2.0)
35.9–204.5	63	1.2 (0.8–2.0)	1.0 (0.5–2.1)
		P for trend‖ = .05	*P* for trend‖ = .94
PhIP, ng/d			
0–0.2	44	1.0 (referent)	1.0 (referent)
0.3–6.5	48	1.1 (0.6–1.7)	1.1 (0.6–1.8)
6.6–22.9	49	1.1 (0.7–1.8)	1.2 (0.7–1.9)
23.0–55.7	57	1.3 (0.8–2.1)	1.4 (0.8–2.3)
55.8–523.1	75	1.7 (1.1–2.8)	1.9 (1.1–3.4)
		P for trend‖ <.001	*P* for trend‖ <.001

*The Spearman correlation coefficients = .76 between 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline (DiMeIQx) and 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx); .60 between 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) and MeIQx; and .43 between PhIP and DiMeIQx.

†OR (odds ratio) was adjusted for age, total energy intake, family history of breast cancer, use of hormone replacement therapy, and waist-to-hip ratio.

‡OR was adjusted for age, total energy intake, family history of breast cancer, use of hormone replacement therapy, and waist-to-hip ratio, as well as the HCAs.

§Quintile cut points were to distribution in the control population.

¶Total number of case patients = 273 and control subjects = 657.

‖Tests for trend were calculated by using the continuous data.

However, if the errors are different (HCA levels are underestimated for case subjects and overestimated for control subjects), it can lead to bias away from the null.

We investigated whether HCAs found in well-done red meats could explain the well-done meat association with breast cancer reported by Zheng et al. *(1)*. In separate models, the ORs were 1.03 (95% CI = 1.01–1.05; *P* for trend <.001) per 10 ng of PhIP (Table 2) and 1.27 (95% CI = 1.05–1.60; *P* for trend = .01) per 10 g of very well-done red meat. With the two variables adjusted for each other, the OR remained 1.03 (95% CI = 1.01–1.06; *P* for trend = .02) per 10 ng of PhIP. In contrast, the OR for very well-done meat decreased to 1.04 (95% CI = 0.80–1.52; *P* for trend = .73) per 10 g of meat, indicating that PhIP may be more strongly associated with risk than very well-done red meat. When the effects of both PhIP intake and grams of very well-done meat were jointly estimated, the errors of those estimates are larger than when these effects were estimated separately, creating larger CIs.

The main advantage of this study is that we used an extensive database created from hundreds of meat samples cooked by different methods to various degrees of doneness. This database was designed to take into account the various cooking techniques used by the U.S. population *(17,19)*. The food-frequency questionnaire was designed specifically to address the HCA hypotheses. It is crucial to obtain very specific information on cooking methods (fry, broil, grill, etc.) and doneness levels (rare, medium, well-done, very well-done, etc.) for different meat types (hamburger patty, steak, roast, etc.) to estimate HCA intake as accurately as possible. To highlight the importance of this approach, one may compare the level of PhIP found in different types of red meat cooked by different techniques *(11)*. For example, 30.0 ng of PhIP/g of meat was measured in a very well-done grilled steak, whereas PhIP was not detected in a very well-done oven-broiled hamburger patty.

One limitation of this study is that HCA values are based on intakes of only three meats (beef steak, hamburger patty, and bacon). These three, however, accounted for more than 60% of the red meat consumed in the population studied *(1)* and contain even more of the HCAs consumed. Certain cooking methods can produce high levels of PhIP in chicken *(20)*, but we could not include HCAs from chicken because doneness information on chicken was not obtained.

The participation rate for this study was 60% for the case subjects and 75% for the control subjects. One possible explanation for the positive association found in this brief communication is that of selection bias. This could happen, for example, if participation rates among case subjects who consumed high levels of HCA were higher than those among case subjects who consumed low levels of HCAs, whereas the participation rates among control subjects who consumed high levels of HCAs were lower than those among control subjects who consumed low levels of HCAs. The participants were, in general, similar to the subcohort of women eligible for the study on baseline risk factors and dietary habits measured *(1)*. The retrospective nature of this study and possibilities of bias in the subject's responses are of concern. However, we obtained similar results for breast cancer risk with total red meat intake in both the prospective and the case–control studies. Fur-

thermore, at the time the study was conducted, we had no reason to believe that the patients with breast cancer, compared with the control subjects, would have given different responses about meat-cooking methods.

Consumption of PhIP may play a role in the development of breast cancer. There is little evidence from this study that either DiMeIQx or MeIQx increases the risk of breast cancer. The result lends some credence to PhIP being a mammary carcinogen. It is important that we further evaluate this finding in other epidemiologic studies that use a detailed assessment of meat-cooking techniques and a comprehensive HCA database.

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NOTES

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